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**Review**

# Methods for obtaining extracts of macroalgae and cyanobacteria, evaluation of their biological activities

## Métodos para la obtención de extractos de macroalgas y cianobacterias, evaluación de sus actividades biológicas

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**ABSTRACT:** The increase in the yield and productivity of crops is one of the challenges that farmers experience today. The inappropriate use of chemical products in agriculture has caused the loss of the fertile layer of the soil, has diminished its biodiversity and has been eliminating the natural enemies of pests. For this reason, it has become necessary to search for other alternatives to achieve this goal, such as the use of macroalgae and cyanobacteria extracts as plant biostimulants. They contain a wide variety of plant growth-promoting substances, such as auxins, cytokinins, betaines, gibberellins, and organic substances such as amino acids, macronutrients, and trace elements that improve crop yield and quality. With this review, it is proposed to address the different methods for obtaining extracts from macroalgae of the genus *Sargassum* spp. and cyanobacteria of the genus *Arthrospira*, as well as the evaluation of their biological activities in physiological processes such as germination, the development of the root system and the yield increase.

**Key words:** biostimulant, growth, crops, phytohormones.

**RESUMEN:** El incremento en el rendimiento y la productividad de los cultivos es uno de los desafíos que experimentan los agricultores en la época actual. La utilización inadecuada de productos químicos en la agricultura ha ocasionado la pérdida de la capa fértil de los suelos, ha disminuido su biodiversidad y ha ido eliminando a los enemigos naturales de las plagas. Por esta razón, se ha hecho necesaria la búsqueda de otras alternativas para lograr este fin, como es el caso de la utilización de los extractos de macroalgas y cianobacterias como bioestimulantes vegetales. Estos contienen una amplia variedad de sustancias promotoras del crecimiento de las plantas, tales como auxinas, citoquininas, betaínas, giberelinas y sustancias orgánicas como aminoácidos, macronutrientes y oligoelementos que mejoran el rendimiento y la calidad de los cultivos. Con esta revisión se propone abordar los diferentes métodos para la obtención de los extractos de macroalgas del género *Sargassum* spp. y de cianobacterias del género *Arthrospira*, así como la evaluación de sus actividades biológicas en procesos fisiológicos como la germinación, el desarrollo del sistema radical y el aumento del rendimiento.

**Palabras clave:** bioestimulantes, crecimiento, cultivos, fitohormonas.

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## INTRODUCTION

Algae are plant organisms, unicellular or multicellular, autotrophic and occupy the first link in the food chain in the aquatic environment. They can inhabit freshwater, saltwater or out of water in humid places, such as coastal rocks. They exist in great variety, have different shapes and colors. They reproduce sexually or asexually by fragmentation or division. According to their size, they are divided into two large groups: macroalgae, the larger ones, and microalgae, the smaller ones (1).

Macroalgae are defined as eukaryotic, photosynthetic, multicellular organisms with an enormous variety of morphologies and life cycles. Some of the characteristics on which the criteria for grouping them are based are cytological (levels of organization), biochemical (pigments, cell wall composition) and reproductive (reproductive cycles and structures) characters. Most species are grouped within the *phylum Chlorophyta* (green), *Rhodophytas* (red) and *Ochrophyta* (brown). In Cuba, a great variety of macroalgae abounds, among which are: *Amphiroa*, *Galaxaura*, *Lobophora*, *Dictyota*, *Halophila* and *Sargassum* (2).

On the other hand, cyanobacteria, which have long been known as blue-green algae, have recently been placed among the gram-negative bacteria by morphological and genetic analyses and are microorganisms whose cells measure only a few micrometers in diameter but whose length is much greater than that of most other bacteria. Among them is Spirulina, belonging to the genus *Arthrospira* and three species of edible Spirulina have been studied for their high nutritional value and potential therapeutic properties, which are *Arthrospira platensis*, *Arthrospira maxima* and *Arthrospira fusiformis* (3). For their development they require $CO_2$, nitrogen, phosphorus, potassium, magnesium and other minor nutrients such as metals. Their dry mass includes between 60-70 % of proteins, carbohydrates, vitamins such as provitamin A, vitamin C, vitamin E, minerals such as iron, calcium, chromium, copper, magnesium, manganese, phosphorus, potassium, sodium and zinc, essential fatty acids. Pigments such as chlorophyll a, phycocyanin and carotenes are also present (4).

In recent years, natural products based on this macroalgae and cyanobacteria have been used as agrochemical substitutes and have acquired great importance due to the benefits they have on crops and the reduced impact they cause to the environment. It has been proven that their application increases certain metabolic and physiological expressions in plants. These products are mainly marketed as biofertilizers because of their high content of macronutrients and micronutrients or as biostimulants because they contain, among other compounds, growth promoting hormones and minerals, as is the case of *Sargassum* and Spirulina extracts (1-4).

In view of the above, the objective of this literature review is to provide an updated overview of the methods used to obtain *Sargassum* and Spirulina extracts, as well as the evaluation of their biological activities.

## GENERAL CHARACTERISTICS OF SPIRULINA (*ARTHROSPIRA* SPP.)

Spirulina, belonging to the genus *Arthrospira* and the family Oscillatoriaceae, is a microscopic blue-green cyanobacterium with a multicellular character, where the blue color comes from the phycocyanin present and the green from the chlorophyll. It derives its name from the spiral nature of its open helix filaments (trichomes). It has become an object of scientific study due to its bioavailability of nutrients, since 85-95 % are assimilable (5). It presents cylindrical cells and a plasma membrane that is surrounded by a pluri-stratified cell wall, which has a series of pores around the trichome, this in turn, is wrapped by a capsule or sheath composed of polysaccharides. The cell wall is formed by soft mucopolysaccharides, which facilitates the assimilation of nutrients (5, 6).

## GENERAL CHARACTERISTICS OF *SARGASSUM* SPP.

*Sargassum* spp. is a genus of macroscopic algae belonging to the Phaeophyta group or brown macroalgae, which have a cell wall consisting of a fibrous skeleton and an amorphous matrix, which is attached to the fibrous skeleton through hydrogen bonds. The term Sargasso groups several species of brown macroalgae within the genus *Sargassum* such as *Sargassum vulgare*, *Sargassum filipéndula*, *Sargassum fluitans*, *Sargassum natans*, *Sargassum cymosum* or *Sargassum wightii*, among others. They are large in size and their structure is divided into the classic rhizoids, stipes and laminae, analogous to the roots, stems and leaves of vascular plants (7).

The algae of the genus *Sargassum* are distributed throughout tropical and temperate zones and contribute significantly to the biomass of many coastal areas. They possess a great capacity for growth and reproduction due, among other factors, to their ability to reproduce vegetatively. Most of the species of this genus recorded for the Cuban coasts have a benthic life form, except *Sargassum fluitans* and *Sargassum natans*, which are pelagic. These two species float in large agglomerates, are characteristic of the Sargasso Sea, mainly in the North Atlantic, and reach the coasts forming small or large upwellings (7, 8).

## METHODS FOR OBTAINING EXTRACTS

Extracts can be obtained by physical, chemical and microbiological processes and can be used in any field of the chemical and medical-pharmaceutical industry (9). The scheme in Figure 1 shows the most significant methods of obtaining extracts (10).

Los extractos se pueden obtener por procesos físicos, químicos y microbiológicos y pueden ser utilizables en cualquier campo de la industria química y médico-farmacéutica (9). En el esquema de la Figura 1 se muestran los métodos más significativos de obtención de los extractos (10).

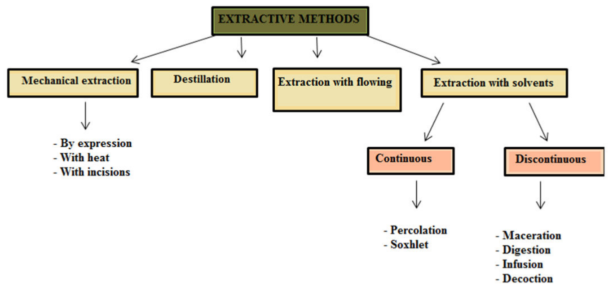


**Figure 1.** Methods used to obtain *Sargassum* and Spirulina extracts

The most widely used method for obtaining extracts for agricultural purposes is solvent extraction, which consists of separating the active principles of the algae by placing it in contact with a solvent or mixture of solvents capable of solubilizing these principles. This extraction can be continuous or discontinuous, being the maceration method the most commonly used in agriculture and is based on soaking the starting material, duly fragmented, in a solvent (water, ethanol, etc.) until it penetrates and dissolves the soluble portions (9).

## CLASSIFICATION OF EXTRACTS

Extracts are concentrated preparations of solid, liquid or intermediate consistency, generally derived from dried starting material, obtained by partially or totally evaporating the solvent in the extractive liquids of plant origin. Depending on the degree of concentration of extractive solvents, extracts can be classified as: liquid, dry, semi-solid or cryoextracts. The most commonly used in agriculture are liquid extracts, which are preparations of the starting material containing alcohol as solvent or preservative or both (9, 11).

## PARAMETERS THAT INFLUENCE THE OBTAINING OF EXTRACTS

It is important to establish extraction parameters to achieve standardization of the process; this will guarantee the quality, yield and efficiency of the final product. The most significant parameters for this purpose are described below (9).

1. **Chemical nature of the starting material:** it is essential to know the properties or characteristics of the metabolite to be extracted (9).

With the study of the species composition and chemical characterization of the *Sargassum influx* in six different localities along the Mexican Caribbean coast, it was obtained that the biochemical composition of sargassum was spatially homogeneous for most of the analyzed components; only the carbon content, the ash metals (particularly Fe and As) and the isotopic composition changed spatially (12).

2. **Solvent:** the optimum solvent is the one that extracts the highest yield of the compound under study. Several studies have been carried out to obtain extracts with different solvents (9).

In a study of the effect of several extracts of *Sargassum* spp. obtained from different solvents: methanol, hexane, dichloromethane and water, used as fertilizer on the plant growth of *Capsicum annum* (Chilli) and *Lycopersicon esculentum* (tomato), it was obtained that the extract with hexane as solvent was more effective to promote the development of seedlings (13). On the other hand, three solvents (distilled water, sodium phosphate with pH 7.4 and $CaCl_2$ 5 %) have been used to obtain phycocyanin from *Arthrospira platensis* and it was found that the highest yield was reached with $CaCl_2$ (14).

3. **Temperature:** the increase in temperature favors extraction; however, high temperatures can lead to leaching of undesirable solutes (9).

In this sense, some authors worked on the optimization of several factors, among them the temperature for obtaining antioxidants from Spirulina extracts, resulting in an optimum temperature of 48 ºC (15). In another research, where the biostimulant potential of hydroalcoholic extracts of *Sargassum* spp. was evaluated with temperature variation, 120 ˚C was the optimum temperature (16).

4. **Solid-liquid ratio:** the appropriate ratio will be the one that achieves to extract the highest amount of the active principle of interest (9).

When studying the effect of *Sargassum tenerrimum* extract on seed germination and growth of tomato plants (Solanum lycopersicum) at concentrations of 0.2, 0.4, 0.6, 0.8 and 1 %, it was obtained that the best results were achieved with the concentration of 0.6 % (17). Similar studies were carried out on radish, with extracts of *Sargassum vulgare* (18) and on *Vigna mungo* and *Vigna radiata* with *Sargassum polycystum* (19).

5. **Particle size:** small particle size favors greater contact between the algae and the solvent (9).

Regarding the analysis of the effect of sample particle size on the phytochemical composition and antioxidant activity of the brown macroalga *Sargassum cristaefolium*, it was obtained that the smallest particle size (< 45 μm) showed the strongest antioxidant activity, confirming the importance of reducing the particle size of macroalgae samples to increase the effectiveness of their biological activity (20).

6. **Medium viscosity:** it is advisable not to use solvents with relatively high viscosity (9).

7. **Agitation speed and extraction time:** these parameters should be adjusted to achieve a higher product yield (9).

## EVALUATION OF THE BIOLOGICAL ACTIVITY OF THE EXTRACTS

The biostimulant effect of macroalgae extracts is due to the combined action of all the components present in these formulations. Among them, plant growth regulators are one of the groups with the highest biological activity, so that many of their effects are hormonal (21).

Cytokinins, auxins, abscisic acid, gibberellins, salicylic acid, polyamines and ethylene have been identified in Sargasso extracts. The most relevant, in terms of quantity and activity, are cytokinins and auxins. Other compounds identified in the extracts that can regulate plant growth are: rhodomorphin, jasmonates, brassinosteroids, strigolactones and betaines. In addition, these extracts possess other bioactive compounds, which can induce defense mechanisms in plants and promote plant growth. For example, polyunsaturated fatty acids (ω-3 and ω-6), amino acids (alanine, glycine, valine, aspartate, glutamate, arginine, threonine), vitamins (B, C, E and K) and sterols (fucoesterol, ergosterol, cholesterol, stigmasterol). Likewise, osmoprotective compounds have been identified that can improve the agronomic response of crops under osmotic stress conditions, such as betaines, proline, sorbitol and mannitol (21, 22).

For all these reasons, the use of sargassum extracts as yield stimulators for crops such as rice (23-26), cowpea (27, 28), radish (18), tomato (29), okra (30) and grapevine (31), among others, has increased.

The biostimulant activity of cyanobacterial extracts and especially Spirulina have been associated with the content of primary metabolites (carbohydrates, proteins and lipids), key amino acids (arginine and tryptophan), and vitamins, osmolytes such as proline and glycinbetaine and polysaccharides (β-glucan). Therefore, identifying and selecting macroalgae and cyanobacteria extracts containing plant hormones, particularly, auxins and cytokinins, which play an important role in promoting plant growth, yield and plant defense response, especially, to abiotic stress, can be considered a growing opportunity for their valorization (32).

The effects that the application of Spirulina has caused in different plant species have been reported by several authors. Thus, in *Amaranthus gangeticus*, it has been found that imbibition of seeds and foliar application of Spirulina extracts increased protein (33) and iron levels in plants (34). Similarly, it was reported that imbibition of *Phaseolus aureus* and *Solanum lycopersicum* L. seeds in extracts of this cyanobacterium increased Zn levels in plants (35).

In beans, foliar application of an aqueous extract stimulated growth, chlorophyll, nitrogen, phosphorus and potassium concentrations, as well as seed quantity and quality (36).

## HORMONAL EFFECTS OF EXTRACTS

As previously stated, sargassum and Spirulina extracts contain plant hormones, hence their effects in promoting plant growth and development (21-32).

- Auxin type

Auxins are a type of phytohormones specialized in different processes at the plant level. Among the most relevant characteristics of auxins are their capacity to induce the formation and elongation of stems at the plant level, promote cell division in callus cultures in the presence of cytokinins, and have the capacity to induce the production of different adventitious roots on leaf tissues and freshly cut stems (37). In 2011, the aqueous extract of *Sargassum johnstonii* was used on tomato (*Lycopersicon esculentum* Mill) seedlings, obtaining that the extract induced the development of adventitious roots (38).

In addition, the influence of the concentration of aqueous extracts of sargassum on the germination and growth of different crops has been investigated. Thus, the effect of different concentrations of an aqueous extract of *Sargassum liebmannii* on the germination of *Pachyrhizus erosus* was studied, and the results showed that treatment of seeds for 24 hours with a concentration of 2 % increased germination by 25 % (39). On the other hand, the application of different concentrations of an aqueous extract of *Sargassum crassifolium* on corn seedlings revealed that the 20 % concentration was the best, significantly increasing the dry mass, height and leaf area of the seedlings (40). Similar results were also obtained with the same concentration and species of Sargassum in *Vigna unguiculata* L. (41). In another study carried out with an aqueous extract of *Sargassum polycystum* on *Vigna mungo* and *Vigna radiata* seedlings, it was found that the concentration of 3 % was the one that promoted the maximum growth of the seedlings (19).

The application of dry flour of Sargassum vulgare to the soil for sowing coriander seeds showed that, in the treatments with a higher concentration of flour, the main root decreased and the adventitious roots increased, which presented a more rigid texture, while in the treatments without flour the root was delicate and fragile (42).

- Cytokinin type

Cytokinins have the ability to stimulate and induce high cell proliferation and division, they usually induce root initiation and elongation and can delay leaf senescence, allowing to stimulate plant photomorphogenic development and play an important role in increasing and generating shoot production at the plant level (21).

When Spirulina was applied directly to soil planted with sunflower, soybean, green beans and peanuts, there were positive effects on plant growth and yield (43).

As for algae, some authors compared the bioactivity in chlorophyll synthesis of the aqueous extract of *Sargassum johnstonii* (dilutions: 0.1; 0.4 and 0.8 %) on cotyledons of *Cucumis sativus*, obtaining an optimal result in the 0.8 % dilution (44).

- Salicylic acid type

Salicylic acid regulates plant growth and development processes and is a signal molecule that activates plant defense mechanisms under environmental stress conditions (21). The use of aqueous extracts of Spirulina and

*Chlorella* spp. improved wheat tolerance to salinity, antioxidant capacity and protein content of the whole grains produced (45).

It was also found that foliar spraying with a Spirulina extract to *Vicia faba* plants at flowering reduced the adverse effects of salinity on plants, stimulating the level of total protein, N, P and K content, as well as photosynthetic activity (46).

Several investigations have demonstrated the effectiveness of sargassum extract applications to stimulate growth and mitigate the adverse effects induced by certain abiotic stresses on plants. Thus, treatment of wheat seeds with an extract of *Sargassum vulgare* stimulated germination in saline medium (47); whereas, foliar spraying with an aqueous extract of *Sargassum muticum* on chickpea seedlings ameliorated the negative impacts of soil salinity through multiple mechanisms including balanced ionic content and stimulation of antioxidant defense. In addition, key amino acids (serine, threonine, proline, and aspartic acid) were identified in the roots, which are responsible for the stress response of plants mediated by this macroalga (48).

## ANTIOXIDANT ACTIVITY

The main antioxidants in algae are phenolic compounds. They are followed by vitamins and carotenoids, which are also found in significant amounts (49).

Phenolic compounds are generally found in plants and vegetables. Within this large group are phenolic acids, polyphenols and flavonoids. These are a series of compounds of great interest, since they have antioxidant capacity, which implies a beneficial effect for our organism and is also interesting from a technological point of view, since they act as natural antioxidants. Regarding the differences between the percentages of phenolic compounds present in algae, the influence of the species, the season of the year and environmental factors such as light, salinity and nutrients should be highlighted (49).

Many studies have been carried out to investigate the content of phenolic compounds in algae. When evaluating the antioxidant activity of three Spirulina extracts (methanol, acetone and hexane). The results showed that these extracts significantly scavenge ABTS and DPPH radicals in a dose-dependent manner. The methanolic extract had higher total phenolic content and antioxidant activity than other extracts (50).

In aqueous and ethanolic extracts of *Spirulina platensis*, antioxidant activity and phytonutrient content were studied, showing that the total phenol, flavonoid and tannin contents were high in the aqueous extract. However, the protein and carbohydrate content was higher in the ethanolic extract (3).

In an optimization study of the extraction process of phenolic compounds from the seaweed *Sargassum fluitans* Børgesen (Børgesen), it was found that the highest amount of total polyphenols (8.66 mg $g^{-1}$ dry mass) was obtained with ethanol 17.75 % in a 1:10 ratio (m/v) and incubation at 50 ºC with shaking for 123.5 min (51).

The use of various organic solvents for the extraction of S. polycystum yielded that the ethanolic extract (70 %) exhibited the highest total phenolic content (627 ± 50.81 mg GAE 100 $g^{-1}$ dry mass) and the highest DPPH scavenging activity (61.4 ± 0.171 %) at the highest concentration tested (3 mg $ml^{-1}$). The methanolic extract, on the other hand, presented the highest total antioxidant capacity (121.00 ± 0.003 mmol $g^{-1}$) (52). However, in a previous study where the antioxidant capacity of *S. aquifolium*, *S. ilicifolium* and S. polycystum was evaluated, it was demonstrated that the extraction with enzymes was superior to those carried out with organic solvents such as methanol, methanol 50 % and ethanol 75 % (53).

Analysis of the carotenoid composition of three Malaysian edible seaweed species (*Eucheuma denticulatum*, *Sargassum polycystum* and *Caulerpa lentillifera*) showed that fucoxanthin was the main carotenoid present in *S. polycystum*, while lutein and zeaxanthin in E*. denticulatum*. For *C. lentillifera*, β-carotene and canthaxanthin were the main carotenoids. Some of the carotenoids, such as rubixanthin, dinoxanthin, diatoxanthin and anteraxanthin, were also tentatively detected in *E. denticulatum* and *S. polycystum*. As for antioxidant activity, S. polycystum (20 %) and *E. denticulatum* (1128 µmol TE $g^{-1}$) showed the highest activity in DPPH and ORAC assays, respectively (54).

These characteristics presented by sargassum extracts have made it possible to increase the antioxidant and antimicrobial capacity of various cultures. Thus, it was demonstrated that foliar spraying of cowpea plants (*Vigna unguiculata* L. Walp) with an extract of *Sargassum swartzii* (3 %) increased their antioxidant capacity (55).

In an investigation aimed at determining the antioxidant activity of *Spirulina platensis* extracts by supercritical carbon dioxide extraction, it was obtained that the extracts contained: flavonoids (85.1 g $kg^{-1}$), ß-carotene (77.8 g $kg^{-1}$), vitamin A (113.2 g $kg^{-1}$) and α-tocopherol (3.4 g $kg^{-1}$), which is largely due to their high antioxidant activity. The main fatty acids in the extracts were palmitic acid (35.32 %), linolenic acid (21.66 %) and linoleic acid (20.58 %) (15).

When evaluating the antioxidant activity of the algae *Ulva fasciata* (Chlorophyta), *Sargassum linifolium* (Phaeophyta) and *Corallina officinalis* (Rhodophyta), the results showed that β-carotene was maximum (3940.12 IU 100 $g^{-1}$) in C. officinalis. DPPH antioxidant activities were highest in *U. fasciata* (81.3 %) followed by *S. linifolium* (79.8 %) and then *C. officinalis* (72.6 %) (56).

All these functional characteristics of macroalgae and cyanobacteria extracts, especially those of sargassum and Spirulina, demonstrate that they constitute a natural alternative that allows reducing the use of agrochemical products in agricultural production systems. Their application as biostimulants helps to improve the agronomic response of different crops, stimulate the nutritional content of the agricultural product and increase the useful life of post-harvest products (21).

## CONCLUSIONS

- Sargassum and Spirulina extracts most commonly used in agriculture are obtained by solvent extraction and maceration.
- These extracts have a wide variety of compounds, such as amino acids, macronutrients, growth regulators and phenols, which improve crop yield and quality and can protect crops against environmental stress conditions.

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